PCT/EP99/07755

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 16 June 2000 (16.06.00)	
International application No. PCT/EP99/07755	Applicant's or agent's file reference K 2737 - sch/msl
International filing date (day/month/year) 14 October 1999 (14.10.99)	Priority date (day/month/year) 14 October 1998 (14.10.98)
Applicant ROMMELAERE, Jean et al.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 May 2000 (15.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Juan Cruz Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
SCHÜSSLER, Andrea
Truderinger Strasse 246
D-81825 München
GERMANY

EINGANGEN
- 3 MRZ. 2000
Erled. .....

I.S. mit Lf

Applicant's or agent's file reference K 2737 - sch/msl	Date of mailing (day/month/year) 01/03/2000 FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/EP 99/ 07755	International filing date (day/month/year) 14/10/1999
Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Sandra De Jong-van Dam

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
K 2737 - sch/msl		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 07755	14/10/1999	14/10/1998

Applicant

DEUTSCHES KREBSFORSCHUNGSZENTRUM et. al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. -

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIO SEARCH REPORT

International application No.
PCT/EP 99/07755

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 19 and 20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the parvovirus vector.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]EP 99/07755

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/864 C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUPONT F ET AL.: "Use of an autonomous parvovirus vector for selective transfer of a foreign gene into transformed human cells of different tissue origins and its expression therein" JOURNAL OF VIROLOGY, vol. 68, no. 3, March 1994 (1994-03), pages 1397-1406, XP002096869 AMERICAN SOCIETY FOR MICROBIOLOGY US figure 1 ---	1-6,9, 10,15, 17-19
X	MAXWELL I H ET AL: "Autonomous parvovirus transduction of a gene under control of tissue-specific or inducible promoters" GENE THERAPY, vol. 3, no. 1, January 1996 (1996-01), pages 28-36, XP000651804 figure 1 --- -/--	1-5,9, 15-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2000	01/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]EP 99/07755

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 13823 A (Z. COMPANY S.A.; ZEICHER M) 23 June 1994 (1994-06-23) page 8, line 36 -page 29	1-6, 9-11,15, 17-20
A	US 5 302 517 A (RHODE S L) 12 April 1994 (1994-04-12) figure 2	1-20
A	COTMORE S & TATTERSALL P: "An asymmetric nucleotide in the parvoviral 3' hairpin directs segregation of a single active origin" EMBO JOURNAL, vol. 13, no. 17, 1994, pages 4145-4152, XP002096870 EYNSHAM, OXFORD GB cited in the application figure 4	1
A	TAM P & ASTELL C R: "Multiple cellular factors bind to cis-regulatory elements found inboard of the 5'palindrome of Minute Virus of Mice" JOURNAL OF VIROLOGY, vol. 68, no. 5, May 1994 (1994-05), pages 2480-2848, XP002096871 AMERICAN SOCIETY FOR MICROBIOLOGY US the whole document	2

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/07755

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9413823 A	23-06-1994	BE 1006437 A	30-08-1994
		AU 5556094 A	04-07-1994
		CA 2151496 A	11-06-1994
		EP 0673430 A	27-09-1995
US 5302517 A	12-04-1994	NONE	

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
K 2737 - sch/msl		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/07755	14/10/1999	14/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/864		
Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/05/2000	08.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Trommsdorff, M Telephone No. +49 89 2399 7361

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/07755

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-11 as originally filed

Claims, No.:

1-20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 19, 20.

because:

☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-18

No: Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Cited documents

The following documents (D) are referred to in this communication; the numbering is the same as in the search report and will be adhered to in the rest of the procedure:

D1: DUPONT F ET AL.: 'Use of an autonomous parvovirus vector for selective transfer of a foreign gene into transformed human cells of different tissue origins and its expression therein' JOURNAL OF VIROLOGY, vol. 68, no. 3, March 1994 (1994-03), pages 1397-1406, AMERICAN SOCIETY FOR MICROBIOLOGY US

D2: WO 94 13823 A (Z. COMPANY S.A.; ZEICHER M) June 1994 (1994-06-23)

D5: COTMORE S & TATTERSALL P: 'An asymmetric nucleotide in the parvoviral 3' hairpin directs segregation of a single active origin' EMBO JOURNAL, vol. 13, no. 17, 1994, pages 4145-4152 EYNSHAM, OXFORD GB cited in the application

2. Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 19 and 20 are directed to methods of treatment of the human/animal body. For the assessment of said claims on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. Thus, said claims were examined based on the alleged effect of the parvovirus vector.

3. Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventiv step or industrial applicability; citations and explanations supporting such statement

3.1. Novelty

Claim 1 is directed to a parvovirus with an excisable DNA having a left terminus

with a minimal origin of replication.
D1 describes the use of parvovirus vectors to selectively transfer and express a foreign gene in specific tissues. Figure 1 (p.1399) shows the structure of the different vectors used (p.1398, 2nd column). The pMVM/P38cat plasmid derived from pMVM contains a CAT reporter gene inserted into the capsid region, downstream of the MVM P38 promoter. Said vector contains an origin of replication derived from MVM and is thus able to replicate autonomously in permissive cells (p.1400, 1st column). Indeed, the results show that after transfection "the recombinant genome (Cat gene) was excised from pBR322 and efficiently replicated" and expressed. However, said vector does not contain a "minimum origin of replication" in its left arm.
Thus, the subject-matter of claim 1 and dependent claims 2-17 is novel over D1 (Art. 33(1)-(2) PCT).

Document D2 also describes parvovirus derived vectors. The LuIII recombinant plasmids contain terminal inverted repeat sequences to permit DNA replication and packaging (p.33, 1st column, 2nd paragraph). However, none of the disclosed vectors contains a left arm with a "minimal origin of replication".
Thus, claims 1-17 are also novel over D2 (Art. 33(1)-(2) PCT).

Claims 18- 20 are directed to the production or use of said parvovirus vector for gene therapy, more specifically in the case of tumour diseases. Since the claimed parvovirus vector is novel, methods of production and use of said vector are also novel (Art. 33(1)-(2) PCT).

3.2. Inventive step
Parvoviral vectors are described in a number of prior art documents as, e.g. D1 and D2 (see above). They are attractive for cancer therapy, because of their oncosuppressive effect (see description p. 1-2). However, when using the conventional parvoviral vectors, as in D1 or D2, the titers of viral particles obtained are low (D1: p.1402, table 1).
The technical problem of the present application is to obtain higher titers of packaged viral particles.
The solution is a parvovirus vector having an excisable DNA with a left arm comprising a "minimal origin of replication".

Indeed, applicants show that when the left arm is modified such as to contain a functional origin of replication, i.e. comprising the consensus sequence of an NS1 nicking site, thousandfold higher titers can be obtained (p.11, example 4 of the description as filed).
This solution could not have been anticipated or derived by the skilled person from the teaching of the prior art. Hence, the parvoviral vectors of claims 1-17 are inventive (Art. 33 (1) and (3) PCT).
Consequently, claims 18-20 directed to methods of producing said vector and methods of use of said vectors are also inventive.

6. **Re Item VIII**
Certain observations on the international application

6.1. The technical feature "minimal origin of replication " has no well-recognized meaning and is only defined by reference to prior art document D5. However, D5 does not give an exact definition of this expression. Since said feature is an essential differentiating feature of claim 1 and in order to clarify the subject-matter for which protection is sought, the "minimal origin of replication" should be further defined by, e.g. adding the precise sequence elements it contains (Art. 6 PCT). For example, from the description (p.3, l.11-23) it is clear that said "minimal origin of replication" must contain a consensus sequence of an NS-1 nicking site, which is preferably CTWWTCA. Thus, said sequence element should be included in claim 1 instead of being only optional in dependent claim 3.

6.2. From the examples given in the description, it appears that all experiments have been carried out using vectors containing internal replication sequences in their right terminus (see examples 1-5). No experiments have been carried out with vectors wherein said IRS are missing. Thus, it is unclear if such vectors would also yield a high titer of viral particles. Consequently, since said sequence could be essential for the invention and in order for the claims to be fully supported by the description, said technical feature should be included in claim 1.

6.3. The expression "usable in a treatment" of claim10 is vague and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Art. 6 PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
K 2737 - sch/msl		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/07755	14/10/1999	14/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/864		
Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/05/2000	08.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Trommsdorff, M Telephone No. +49 89 2399 7361

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/07755

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-11 as originally filed

Claims, No.:

1-20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 19, 20.

because:

☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-18

No: Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. **Cited documents**

The following documents (D) are referred to in this communication; the numbering is the same as in the search report and will be adhered to in the rest of the procedure:

D1: DUPONT F ET AL.: 'Use of an autonomous parvovirus vector for selective transfer of a foreign gene into transformed human cells of different tissue origins and its expression therein' JOURNAL OF VIROLOGY, vol. 68, no. 3, March 1994 (1994-03), pages 1397-1406, AMERICAN SOCIETY FOR MICROBIOLOGY US

D2: WO 94 13823 A (Z. COMPANY S.A.; ZEICHER M) June 1994 (1994-06-23)

D5: COTMORE S & TATTERSALL P: 'An asymmetric nucleotide in the parvoviral 3' hairpin directs segregation of a single active origin' EMBO JOURNAL, vol. 13, no. 17, 1994, pages 4145-4152 EYNSHAM, OXFORD GB cited in the application

2. **Re Item III**

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 19 and 20 are directed to methods of treatment of the human/animal body. For the assessment of said claims on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. Thus, said claims were examined based on the alleged effect of the parvovirus vector.

3. **Re Item V**

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

3.1. Novelty

Claim 1 is directed to a parvovirus with an excisable DNA having a left terminus

with a minimal origin of replication.
D1 describes the use of parvovirus vectors to selectively transfer and express a foreign gene in specific tissues. Figure 1 (p.1399) shows the structure of the different vectors used (p.1398, 2nd column). The pMVM/P38cat plasmid derived from pMVM contains a CAT reporter gene inserted into the capsid region, downstream of the MVM P38 promoter. Said vector contains an origin of replication derived from MVM and is thus able to replicate autonomously in permissive cells (p.1400, 1st column). Indeed, the results show that after transfection "the recombinant genome (Cat gene) was excised from pBR322 and efficiently replicated" and expressed. However, said vector does not contain a "minimum origin of replication" in its left arm.
Thus, the subject-matter of claim 1 and dependent claims 2-17 is novel over D1 (Art. 33(1)-(2) PCT).

Document D2 also describes parvovirus derived vectors. The LuIII recombinant plasmids contain terminal inverted repeat sequences to permit DNA replication and packaging (p.33, 1st column, 2nd paragraph). However, none of the disclosed vectors contains a left arm with a "minimal origin of replication".
Thus, claims 1-17 are also novel over D2 (Art. 33(1)-(2) PCT).

Claims 18- 20 are directed to the production or use of said parvovirus vector for gene therapy, more specifically in the case of tumour diseases. Since the claimed parvovirus vector is novel, methods of production and use of said vector are also novel (Art. 33(1)-(2) PCT).

3.2. Inventive step
Parvoviral vectors are described in a number of prior art documents as, e.g. D1 and D2 (see above). They are attractive for cancer therapy, because of their oncosuppressive effect (see description p. 1-2). However, when using the conventional parvoviral vectors, as in D1 or D2, the titers of viral particles obtained are low (D1: p.1402, table 1).
The technical problem of the present application is to obtain higher titers of packaged viral particles.
The solution is a parvovirus vector having an excisable DNA with a left arm comprising a "minimal origin of replication".

Indeed, applicants show that when the left arm is modified such as to contain a functional origin of replication, i.e. comprising the consensus sequence of an NS1 nicking site, thousandfold higher titers can be obtained (p.11, example 4 of the description as filed).
This solution could not have been anticipated or derived by the skilled person from the teaching of the prior art. Hence, the parvoviral vectors of claims 1-17 are inventive (Art. 33 (1) and (3) PCT).
Consequently, claims 18-20 directed to methods of producing said vector and methods of use of said vectors are also inventive.

6. Re Item VIII
Certain observations on the international application

6.1. The technical feature "minimal origin of replication " has no well-recognized meaning and is only defined by reference to prior art document D5. However, D5 does not give an exact definition of this expression. Since said feature is an essential differentiating feature of claim 1 and in order to clarify the subject-matter for which protection is sought, the "minimal origin of replication" should be further defined by, e.g. adding the precise sequence elements it contains (Art. 6 PCT). For example, from the description (p.3, l.11-23) it is clear that said "minimal origin of replication" must contain a consensus sequence of an NS-1 nicking site, which is preferably CTWWTCA. Thus, said sequence element should be included in claim 1 instead of being only optional in dependent claim 3.

6.2. From the examples given in the description, it appears that all experiments have been carried out using vectors containing internal replication sequences in their right terminus (see examples 1-5). No experiments have been carried out with vectors wherein said IRS are missing. Thus, it is unclear if such vectors would also yield a high titer of viral particles. Consequently, since said sequence could be essential for the invention and in order for the claims to be fully supported by the description, said technical feature should be included in claim 1.

6.3. The expression "usable in a treatment" of claim10 is vague and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Art. 6 PCT).

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(54) Title: PARVOVIRUS VECTORS AND THEIR USE

(57) Abstract

This invention relates to a parvovirus vector having a parvovirus DNA excisable from the vector DNA in a parvovirus–permissive cell, the parvovirus DNA having a left terminus which comprises a parvovirus minimal origin of replication, and a system comprising the parvovirus vector. Furthermore, this invention concerns a method of producing the parvovirus vector, parvoviral particles as well as their use.

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Parvovirus Vectors and Their Use

The present invention relates to parvovirus vectors and systems containing the same. Furthermore, this invention concerns a method of producing the parvovirus vectors and their use.

Parvovirus designates a genus of the virus family Parvoviridae. The parvovirus genus comprises a number of small, icosaedric viruses that can replicate in the absence of a helper virus. Parvovirus contains a single-stranded DNA having a size of about 5.000 bp. At the 3' and 5' ends of the DNA there is one palindromic sequence each. The DNA codes for two capsid proteins, VP1 and VP2, as well as for two regulatory non-structure proteins, NS-1 and NS-2. The expression of the latter proteins is controlled by a promoter, P4, while a promoter, P38, which is transactivated by NS-1, is responsible for the expression of the capsid proteins.

Parvoviruses are usually well-tolerated by populations of their natural host, in which they persist without apparent pathological signs. This is due to both the protection of foetuses and neonates by maternal immunity, and the striking restriction of parvovirus replication to a narrow range of target proliferating tissues in adult animals. This host tolerance concerns especially rodent parvoviruses, for example the minute virus of mice (MVM) and H-1 virus in their respective natural hosts, namely mice and rats. In addition, humans can be infected with the latter viruses, without any evidence of associated deleterious effects from existing epidemiological studies and clinical trials. On the other side, it is known that certain parvoviruses, and especially rodent parvoviruses, are both oncotropic, i.e. accumulate preferentially in neoplastic versus normal tissues, and oncosuppressive, i.e. have a tumorsuppressive effect towards

tumor cells, in various animal models. At least part of the oncosuppressive effect is thought to be due to a direct oncolytic action mediated by the parvoviral NS1 product. This oncosuppressive effect was also demonstrated against human tumor cells transplanted in recipient animals.

This could be utilized for treating tumors. For this purpose, it is, however, desirable to modify parvoviruses in well-calculated fashion, i.e. give them new properties, e.g. to express therapeutic genes, and provide a great quantity thereof. The former appears to be possible by a parvovirus vector in which parvovirus DNA converted into a double strand is ligated with a vector DNA and the parvovirus DNA region coding for the capsid proteins is replaced by exogeneous DNA. Following the transfection of parvovirus-permissive cells, such a parvovirus vector is subjected to the excision of the parvovirus DNA and its amplification and packaging, respectively, into parvoviruses (cf. Russell, S.J. et al., Journal of Virology, 1992, 2821-2828). However, the yield of parvovirus DNA which is amplified and packed, respectively, is unsatisfactory.

Therefore, it is the object of the present invention to provide a composition by which a great quantity of packed, optionally modified, parvovirus DNA can be produced.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to a parvovirus vector having a parvovirus DNA which can be excised from the vector DNA in a parvovirus-permissive cell, the parvovirus DNA having a left terminus which comprises a minimal parvovirus origin of replication.

The present invention is based on the applicant's finding that in parvovirus-permissive cells a parvovirus present in a parvovirus vector can be excised therefrom and be replicated

when its left terminus comprises a minimal parvovirus origin of replication.

The expression "parvovirus-permissive cell" comprises any cells in which a parvovirus genome can be amplified and packed into infectious viral particles. Examples of such cells are established cell lines of mice, e.g. A9 cells, of human origin, e.g. NB-E-, NB324K, 293 T cells, and of monkey cells, e.g. COS cells.

The expression "left terminus" refers to the 3' end of a parvovirus DNA available as a double strand. As mentioned above, a parvovirus DNA is usually single-stranded. However, such a DNA can be converted into a double strand by common methods. In this form it is then ligated directly or indirectly, e.g. via a linker, with a conventional vector DNA. According to the invention, the left terminus of the parvovirus DNA includes a minimal parvovirus origin of replication. For the definition of a minimal parvovirus origin of replication, reference is made to Cotmore and Tattersall, EMBO J. 13, 1994, 4145. It comprises the consensus sequence of an NS-1 nicking site. The consensus sequence is preferably CTWWTCA, W representing any nucleotide. . For the provision of a minimal parvovirus origin of replication at the left terminus of the parvovirus DNA it is favorable to extend the left terminus by an inverted repeat of the unique sequence located immediately downstream from the 3' terminal palindrome of the parvovirus DNA. A person skilled in the art is familiar with processes necessary for this purpose. Reference is made to Maniatis et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 1982, by way of supplement.

As far as the "right terminus", i.e. the 5' end, of the parvovirus DNA available as a double strand is concerned, it may be the naturally occurring 5' terminus of a parvovirus DNA. It may be favorable for the 5' terminus to have internal replication sequences (IRS). They are found e.g. in the RsalA (4431-4579) and RsalB (4579-4662) fragments of the DNA of the

parvovirus MVMp (cf. Tam and Astell, Virology 193, 1993, 812-824, and J. Virol. 68, 1994, 2840-2848).

In a preferred embodiment, the parvovirus DNA originates from a mammalian parvovirus, particularly a rodent parvovirus, very especially from MVM or H-1. Both rodent parvoviruses are described in the literature (cf. Astell et al.; J. Virol. 57, 1986, 656-669; Rhode and Paradiso, J. Virol. 45, 1983, 173-184; Faisst et al., J. Virol. 69, 1995, 4538-4543). It may be favorable for the parvovirus DNA to comprise a combination of DNA sequences of various parvoviruses, e.g. of mammalian parvoviruses, especially rodent parvoviruses, very especially MVM, H-1 KRV and/or LuIII. It may be particularly advantageous for the parvovirus DNA to originate from H-1 and for its left terminus to comprise a minimal parvovirus origin of replication of MVM.

According to the invention the parvovirus DNA may include an exogeneous DNA. This DNA may be inserted such that it can be expressed. For this purpose, it is favorable for it to be under the control of the parvovirus promoter P38, i.e. it partially or fully replaces the parvovirus DNA region coding for the capsid proteins. An exogeneous DNA is understood to mean any DNA. This may be e.g. an expression element such as a promoter or an enhancer, or a DNA coding for a diagnostic or therapeutic polypeptide. The latter polypeptide is particularly a cytokine, such as a lymphokine, an interleukin or a "colony stimulating factor", a chemotactic polypeptide, such as a polypeptide suitable for attracting monocytes, e.g. MCP-1, or a toxin.

According to the invention the parvovirus DNA may also include deletions of specific parts, e.g. regulatory elements, such as promoters, promoter elements, or genes coding for non-structural proteins. Instead of these deletions an exogenous DNA may be inserted.

Parvovirus vectors of choice fulfilling above conditions are

exemplified below with pdBMVp, pMVM+, phH1, phH1Δ800, phH1Δ800MCP-1, phH1Δ800MCP1Δ3' and phH1Δ800hIL2 (cf. examples 1-3). These parvoviurs vectors have been deposited at DSMZ (Deutsche Sammlung für Mikroorganismen und Zellkulturen) on July 9, 1998 under the following DSM numbers: pdBMVp (DSM 12300), pMVM+ (DSM 12301), phH1Δ800 MCP-I (DSM 12302), phH1Δ800hL2 (DSM 12303), phH1Δ800MCP-1Δ3' (DSM 12304), phH1Δ800 (DSM 12305), phH1 (DSM 12306).

According to the invention the parvoviral genome produced from a parvovirus vector may be packaged in the form of a parvoviral particle. Such a particle is designated to as parvovirus particle and obtainable by common methods. If the parvovirus vector harbors no substitution in essential parvovirus coding and regulatory sequences, it will be an obvious choice to transfect the parvovirus vector only in cells which are parvovirus permissive. Examples of such cells are SV 40-transformed monkey kidney cells, such as COS, or SV40-transformed human kidney cells, such as NB-E, NB324K and 293T, e.g. 293T/17 and A9 mouse cells. Parvovirus vector and parvoviral
particles may then be isolated from the cells.

If the parvovirus vector lacks part or all of the parvovirus DNA region coding for the parvovirus capsid proteins, it will be necessary to transfect the parvovirus vector in parvovirus-permissive cells which simultaneously express the capsid proteins of a parvovirus when parvoviral particles have to be produced. The cells may be the above cells which are transfected with a helper plasmid that permits the expression of the capsid proteins of a parvovirus. The VP proteins may also be provided by capsid genes stably integrated in the cellular genome and constitutively or inducibly expressed.

As far as the sequence coding for the structural proteins (VP) are concerned, it was discovered that certain viral sequences located in the 3' part of the genes coding for the VP proteins should be maintained in the parvovirus vector in order to

obtain high titers of parvoviral particles. These sequences are not or only barely affected by deletions in the VP coding region that do not exceed approximately 800 nt starting from the ATG corresponding to the translation initiation site of the viral VP2 protein. According to the invention these sequences should be maintained if high titers of parvoviral particles have to be produced.

It may be favorable for the helper plasmid mentioned above to contain an SV40 or polyoma virus origin of replication and for the cells to express an SV40 or polyoma large T antigen. Examples of such helper plasmids are p[BK]CMV-VP and p[BK]P38-VP that are based on pBK-CMV (Strategene) and encode H-1 capsid proteins. The helper plasmids pCMVVP(MVM) and pP38VP(MVM) are based on the vector pcDNAI/Amp (Invitrogen Corporation) and can provide MVMp capsid proteins for packaging. In these constructs, the parvovirus capsid protein-coding sequences are under the transcriptional control of the human cytomegaloviurs (CMV) immediate early promotor (p[BK]CMV-VP, pCMVVP(MVM)) or the P38 parvovirus promotor (p[BK]P38-VP, pP38VP(MVM)). COS and 293 T cells can be mentioned as examples of cells which express an SV40 large T antigen. The transfection of cells expressing an SV40 large T antigen with a helper plasmid containing an SV40 origin of replication usually results in the transient expression of parvovirus capsid proteins at an extremely high level.

Furthermore, a stable expression of parvovirus capsid proteins may be advantageous. Suitable for this purpose are also the above cells, particularly 293 T cells, which are stably transfected with a helper plasmid, such as a derivative of the above-mentioned helper plasmids. It may be appropriate for the cell to have stably inserted VP coding genes under control of an inducible promotor (in particular the parvoviral P38 promotor) or a strong constitutive promotor (in particular the human or mouse CMV immediate early promotor). Above cells engineered so as to sustain a stable expression of parvovirus capsid proteins also represent a subject matter of the present

invention. A person skilled in the art is familiar with transfection methods by which the transient or stable expression of parvovirus capsid proteins is obtained. Cells which permit a stable expression of the capsid proteins of a parvovirus also represent a subject matter of the present invention.

Another subject matter of the present invention relates to a system comprising an above parvovirus vector and a cell expressing capsid proteins of parvovirus. It is favorable for the expression of the capsid proteins to be controlled by a helper plasmid containing an SV 40 origin of replication and for the cell to express an SV40 large T antigen. It may also be advantageous for the cell to stably express the capsid proteins of parvovirus, it being preferred when the DNA coding for the capsid proteins is controlled by the P38 parvovirus promoter.

Parvovirus vectors according to the invention distinguish themselves in that they permit higher levels of amplification of the parvovirus genomes that are excised from the parvovirus vectors. Moreover, the above-mentioned packaging cell lines (e.g. monkey COS, 293T) are highly susceptible to transfection by the convenient and cost-sparing Calciumphosphate coprecipitation techniques or DEAE-DEXTRAN and allow the use of shuttle helper plasmids of the type discussed above. The combination of the described changes in parvovirus vector and packaging systems greatly improves the yields of parvovirus vector (parvovirus DNA insert) production giving up to 1000 times higher titers of infectious parvoviral particles as compared with the conventional parvovirus vectors packaging system, in particularly those described in Russell, S.J. et al., above. This represents a great advantage, particularly as regards time and costs. Parvovirus vector and parvoviral particles produced according to the invention are suitable for gene therapy in the best possible way. Especially a gene therapy is indicated in the case of tumor or viral diseases because of the possibility of expressing the cytotoxic viral

protein NS-1 together with a therapeutic polypeptide, particularly cytokines.

The below examples explain the invention.

Example 1: Construction of the parvoviral vectors pdBMVp, pMVM+ and phH1 and the derivative empty parvovirus vector phH1Δ800 according to the invention

Construction of pdBMVp: The vector pdBNco was constructed by putting *Nco*l linkers into the *Sma*I site of pUC19 and then ligating the *Nco*I dimer bridge (dB) fragment from pLEB711 [Cotmore, S.F. and Tattersall, P. (1992). Journal of Virology 66; 420-431] into the resulting *Sma*I site. pdBNco was then linearized with *Bam*HI (in the pUC polylinker) and then partially digested with *Pme*I. The ends of these partials were filled in and ligated together, allowing the isolation of pdB-BP-drop, which is pdBNco deleted for the sequence between the *Bam*HI site in the polylinker and the *Pme*I site in the insert nearest to *Bam*HI. This
procedure destroyed these *Bam*HI and the *Pme*I sites, leaving the remaining *Pme*I in the insert unique. pdB-BP-drop was then digested with *Sap*I (in the plasmid) and *Xba*I (in the polylinker), filled in, and ligated back together to form pdB-SX-drop, just to remove a non-essential part of the plasmid, and to render several sites within the final construct unique. To obtain the final construct, the *Pme*I to *Aat*II fragment of pdB-SX-drop was replaced with the *Pme*I to *Aat*II fragment of the second generation infectious clone pMVM [Gardiner, E.M. and Tattersall, P.(1988) Journal of Virology 62: 1713-1722]. The resulting third generation plasmid is the "dimer bridge" super-infectious clone of MVMp called pdBMVp.

pMVM+ is a spontaneous deletion mutation of pdBMVp missing the MVMp sequences from 4985-5003.

pH1 (infectious clone) consists of the *Sal*I-*Nde*I fragement of

pSR19 [Faisst et al., J. Virol. 69, 4538-4543 (1995)] containing nt 11 to nt 5110 of H-1 (EMBL GenBank#X01457) into the *Nde*I and *Sal*I sites of pUC19 from which the *Hind*III site had been destroyed. phH1 was constructed by replacing the 1386 bp *Hae*II fragment of pH1 by the corresponding fragment of MVM+ containing the dimer bridge, P4 promotor and 995 nt of MVM NS1/NS2 coding region.

Thus the parvovirus DNAs carried by pdBMVp, pMVM+ and phH1 contain a MVM-minimal origin of replcation at the left (3') terminus of the viral genome and are able to provide high amounts of infectious virus upon transfection of monkey COS or 293 T cells as compared with convential parental vectors (pMVM and pSR19) and those described in Russell et al. (1992) which are deprived of a full minimal origin of DNA replication (for instance pMM984). pdBMVp, pMVM+ and phH1 infectious clones are the starting material for the construction of parvovirus DNA containing or not foreign DNA.

For the convenient insertion of transgenes under control of the parvovirus H-1 P38 promoter, a modified parvovirus DNA was constructed from the DNA phH1, whereby the VP2 translation initiation signal (ATG) and approximately 800 nt from the downstream VP sequence were eliminated and replaced by an ochre termination signal (TAA) in frame with VP1, followed by a multiple sequence (CGC CTA GTA CTC GAG CTC TTC GAA GCG GCC GCG GAT CCG ATC GCC TAG GCC CGG GTA TCG AT). More precisely, starting from position nt 2791 of phH1 [numbering according to EMBL/GenBank#X01457, Rhode and Paradiso, (1983). Journal of Virology 45, 173-184], 806 nucleotides were replaced by the above described termination signal and multiple cloning site. This created the empty parvovirus vector phH1Δ800 according to the invention.

Example 2: Construction of parvovirus vectors phH1Δ800MCP-1 andphH1Δ800MCP-1Δ3' according to the invention

The human JE (MCP-1) cDNA [Rollings et al., Mol.Cell.Biol. 4687-4695 (1989)] was obtained from the American Type Culture Collection (ATCC, nr. 61365). The full length cDNA was isolated by PCR using a forward primer containing a *Hind*III site (CTAAGCTTAGCATGAAAGTCTCTGCC) and a reverse primer with an incorporated *Hpa*I site (GCGTTAACTAATAGTTACAAAATAT). After digestion with *Sac*I and *Hpa*I, tha 701 bp PCR fragment was cloned between the *Sac*I and the *Sma*I restriction sites of phHΔ800, to create phH1Δ800MCP-1 according to the invention. The MCP-1 cDNA deprived of its 3' untranslated region (3'UTR) was amplified using the same forward primer and the reverse primer (GCGTTAACTTCAAGTCTTCGGAGTT) with an incorporated *Hpa*I site. After digestion with *Sac*I and *Hpa*I, the 355 bp PCR fragment was cloned between the *Sac*I and *Sma*I restriction sited of phHΔ800 to generate phH1Δ800MCP-1Δ3'. Both vector DNAs achieve high titers of parvoviral particles when parvoviral capsid proteins are simultaneously expressed from a helper plasmid as described above.

Example 3: Construction of the parvovirus vectors phH1Δ800HIL2 according to the inv ntion

The cDNA coding for human IL2 deprived of its 3' untranslated region was excised from the plasmid M13TG5317 (Transgene, Strasbourg) by hydolysis with *Sal*I, and inserted in the *Sal*I site of pBluescript SK+ ,(EMBL/GenBank#X52325) giving phIL2. phIL2 was cut with *Xho*I and *Bam*HI and the 539 bp fragment was inserted in the *Xho*I and *Bam*HI hydrolysed empty parvovirus vector phH1Δ800 (see example 1), generating the human IL2 expressing parvovirus vector phHΔ800hIL2, from which parvovirus DNA and parvoviral particles can be produced.

Example 4: Production of high-titer stocks of parvoviral particles

The genes coding fot the structural proteins of parvovirus H-1 or MVMp under control of the genuine parvoviral promotor P38 or the human CMV immediate early promoter are cloned in the shuttle vector pBK-CMV (Stratagene) or pcDNAI/Amp (Invitrogen), both containing an SV40 origin of replication, this gives rise to the helper plasmids p[BK]P38-VP and p[BK]CMV-VP, which provide H-1 capsid proteins, or pCMVVP(MVM) and pP38VP(MVM), which provide MVMp capsids. 293T cells are transfected with one of the VP-expressing helper plasmids and one of the above parvovirus vectors according to the invention. Parvoviral particles are recovered from the cells and titered by a filter hybridization technique [Russell et al., 1992]. From the parvovirus vectors described in examples 2-3, titers of up to 10^8 replication units of parvoviral particles (described in the examples 2-3) per ml of crude extract can be obtained in this way.

Claims:

1. A parvovirus vector having parvovirus DNA excisable from the vector DNA in a parvovirus-permissive cell, wherein the parvovirus DNA has a lft terminus which comprises a parvovirus minimal origin of replication.

2. The arvovirus vector according to claim 1, characterized in that the right terminus of the parvovirus DNA comprises internal replication sequences.

3. The parvovirus vector according to claim 1 or 2, characterized in that the parvovirus minimal origin of replication comprises the consensus sequence of an NS1 nicking site, particularly CTWWTCA.

4. The parvovirus vector according to any one of claims 1 to 3, characterized in that the parvovirus DNA originates from a mammalian parvovirus.

5. The parvovirus vector according to any one of claims 1 to 3, characterized in that the parvovirus DNA is a rodent parvovirus.

6. The parvovirus vector according to claim 5, characterized in that the rodent parvovirus is MVM or H-1.

7. The parvovirus vector according to any one of claims 1 to 3, characterized in that the parvovirus DNA comprises a combination of DNA sequences of various parvoviruses.

8. The parvovirus vector according to claim 7, characterized in that the parvovirus DNA originates from H-1 and its left terminus comprises a minimal parvovirus origin of replication of MVM.

9. The parvovirus vector according to any one of claims 1 to 8, characterized in that the parvovirus DNA region coding

for the capsid proteins is partially or fully replaced by an exogeneous DNA.

10. The parvovirus vector according to claim 9, characterized in that the exogeneous DNA codes for a polypeptide usable in a treatment.

11. The parvovirus vector according to claim 10, characterized in that the polypeptide is a cytokin or a toxin.

12. The parvovirus vector according to claim 11, characterized in that the cytokin is a chemotactic polypeptide.

13. The parvovirus vector according to claim 12, characterized in that the chemotactic polypeptide is MCP-1.

14. The parvovirus vector according to any one of claims 1 to 13, characterized in that it is present as parvoviral particle.

15. A system comprising the parvovirus vector according to any one of claims 9 to 13 and a cell expressing the capsid proteins of parvovirus.

16. The system according to claim 15, characterized in that the expression of the capsid proteins is controlled by a helper plasmid containing an SV40 origin of replication and the cell expresses an SV40 large T antigen.

17. The system according to claim 15, characterized in that the DNA coding for the capsid proteins is under the control of the parvovirus promoter P38.

18. A method of producing the parvoviral particle according to claim 14, comprising the transfection of a parvovirus-

permissive cell with a parvovirus vector according to any one of claims 9 to 13, the cell expressing the capsid proteins of a parvovirus, and the isolation of the parvoviral particle.

19. Use of the parvovirus vector according to any one of claims 9 to 14 for gene therapy.

20. Use according to claim 19, characterized in that the gene therapy is carried out in the case of tumor diseases.

SEQUENCE LISTING

<110> Deutsches Krebsforschungszentrum

<120> Parvovirus Vectors and Their Use

<130> K2563EU

<140> EP 98 119 409.5
<141> 1998-10-14

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<170> PatentIn Ver. 2.1

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62

INTERNATIONAL SEARCH REPORT

Int[illegible]pplication No
PCT/EP 99/07755

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/864 C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUPONT F ET AL.: "Use of an autonomous parvovirus vector for selective transfer of a foreign gene into transformed human cells of different tissue origins and its expression therein" JOURNAL OF VIROLOGY, vol. 68, no. 3, March 1994 (1994-03), pages 1397-1406, XP002096869 AMERICAN SOCIETY FOR MICROBIOLOGY US figure 1 ---	1-6,9, 10,15, 17-19
X	MAXWELL I H ET AL: "Autonomous parvovirus transduction of a gene under control of tissue-specific or inducible promoters" GENE THERAPY, vol. 3, no. 1, January 1996 (1996-01), pages 28-36, XP000651804 figure 1 --- -/--	1-5,9, 15-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2000	01/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 13823 A (Z. COMPANY S.A.; ZEICHER M) 23 June 1994 (1994-06-23) page 8, line 36 -page 29	1-6, 9-11,15, 17-20
A	US 5 302 517 A (RHODE S L) 12 April 1994 (1994-04-12) figure 2	1-20
A	COTMORE S & TATTERSALL P: "An asymmetric nucleotide in the parvoviral 3' hairpin directs segregation of a single active origin" EMBO JOURNAL, vol. 13, no. 17, 1994, pages 4145-4152, XP002096870 EYNSHAM, OXFORD GB cited in the application figure 4	1
A	TAM P & ASTELL C R: "Multiple cellular factors bind to cis-regulatory elements found inboard of the 5'palindrome of Minute Virus of Mice" JOURNAL OF VIROLOGY, vol. 68, no. 5, May 1994 (1994-05), pages 2480-2848, XP002096871 AMERICAN SOCIETY FOR MICROBIOLOGY US the whole document	2

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 99/ 07755

Box I Observations wher certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 19 and 20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the parvovirus vector.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No
PCT/EP 99/07755

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9413823 A	23-06-1994	BE 1006437 A	30-08-1994
		AU 5556094 A	04-07-1994
		CA 2151496 A	11-06-1994
		EP 0673430 A	27-09-1995
US 5302517 A	12-04-1994	NONE	